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10/692,918	10/24/2003	Frank Grosveld	CARP0015-101	9062

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EXAMINER	
SINGH, ANOOP KUMAR	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/692,918	GROSVELD, FRANK	
	Examiner	Art Unit	
	ANOOP SINGH	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 June 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,7,10,11,33-36,39,41,43 and 44* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 7, 10-11, 33-36, 39, 41, 43 and 44* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Finality of the previous office action of January 5, 2009 has been withdrawn and the prosecution on the merit has been reopened in view of new grounds of rejections.

Applicants' amendment to the claims filed June 2, 2009 has been received and entered. Applicants have amended claims 1, 7, 10, 33, 39 and 41, while claims 2-6, 8-9, 12-32, 37-38, 40, 42 have been canceled. Applicants have also added claims 43-44 are generally directed to elected invention.

Claims 1, 7, 10-11, 33-36, 39, 41, 43 and 44 are pending in this application.

Election/Restrictions

Applicant's election with traverse of group I in the response filed dated April 27, 2006 was acknowledged. The traversal was on the grounds that Group I and Group II-III should be examined together because search for invention of Group I would be coextensive with Group II and III. In addition, applicants asserted that only method of Group I would be required to make the antibody recited in Groups II and III. Applicant's arguments for examining elected method group with the product claims were not persuasive for the reasons of record (see office action dated 2/12/2007). Therefore, the requirement for restriction was deemed proper, maintained and made FINAL. Claims 1, 7, 10-11, 33-36, 39, 41, 43 and 44 are under consideration.

Priority

It is noted that applicants have previously relied on the post filing art of Janessens et al (Proc. National Academy of Science, 2006, 15130-15130, art o record) for the enabling support of instant application that is a continuation (CON) of PCT/IB02/02303 filed on 04/24/2002 which claims benefit from application GB0110029.6 filed in Great Britain on 4/24/2001. It is in this context, while comparing the method disclosed in different application with the post filing art presented by applicants, Examiner noted that claims are not enabling to produce single heavy chain antibody in a transgenic nonhuman mammal disclosed in provisional application filed in

GB. However, applicants' arguments that claimed method does not require loxP site and US Patent 10/29/1996 describes the isotope switching using switch regions is persuasive. Therefore, the effective filing date for instant claims 1, 7, 10-11, 33-36, 39, 41, 43 and 44 is 04/24/2001.

Withdrawn-Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 7, 10-11, 33, 39, 41 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Applicant's amendments to the claims limiting to naturally occurring VHH coding sequence and deletion of VH exon comprising VH coding sequence that has been mutated to be the same as the respective Camelid coding sequence is persuasive. Therefore, previous rejection is hereby withdrawn. Applicants' arguments with respect to the withdrawn rejections are thereby rendered moot.

New Grounds of Claim Rejections -35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 7, 10-11, 33, 39, 43 and 44 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for

A method for the production of a single heavy chain antibody in a transgenic mouse comprising expressing a heterologous VHH heavy chain locus in said mouse specifically in B cells in response to antigen challenge, wherein the VHH heavy chain locus is integrated into the non-human mammal's genome and said VHH heavy chain locus comprises in germ-line configuration: (a) at least one VHH exon, at least one-D exon and at least one-J exon, wherein

the VHH exon, the D exon and the J exon are capable of recombining to form VDJ coding sequence, and wherein the VHH exon comprises a naturally occurring llama VHH coding sequence, and (b) a constant heavy chain region comprising at least one Cμ constant heavy chain gene and at least one of Cγ, Cα, Cε, or Cδ constant heavy chain gene, wherein each of said at least one constant heavy chain gene, when expressed, does not express a functional CH1 domain, (c) a regulatory sequence providing for expression of the VHH heavy chain locus specifically in B cells, said method comprising:

1) immunizing said mammal with an antigen and

2) isolating single heavy chain antibody against said antigen from said mouse,

does not reasonably provide enablement for a method for producing single chain antibody in any other nonhuman mammal. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

The office has analyzed the specification in direct accordance to the factors outlines in *In re Wands*. MPEP 2164.04 states: "[W]hile the analysis and conclusion of a lack of enablement are based on factors discussed in MPEP 2164.01(a) and the evidence as whole, it is not necessary to discuss each factor in written enablement rejection." These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled.

Applicant's arguments filed on June 2, 2009, have been fully considered but they are persuasive in parts. As an initial matter, applicants' amendments to the base claims to recite that the VHH loci is integrated in the mammal's genome obviates the grounds for rejection. Applicants' argument that specific elements disclosed by Janessens et al (Proc. National Academy of Science, 2006, 15130-15130, art of record) that was relied for enabling support is same as element disclosed in the instant specification was found persuasive. Examiner would agree that two llama VHHs that are introduced having characteristic amino acids at positions 42, 49, 50, and 52 are naturally occurring. However, applicants' arguments and amendments are not commensurate with the scope of the claims. It is noted that as amended claims are broad and read on naturally occurring VHH coding sequence of any specie in producing single heavy chain antibody in any nonhuman transgenic mammal. Applicants are not enabled for such a breadth for the reasons discussed in this office action.

The claims are directed to methods for producing single heavy chain antibody in a nonhuman mammal by expressing a heterologous VHH locus comprising VHH exon, D, J exon , a constant heavy chain comprising at least one constant heavy chain gene comprising at least Cμ and at least one of Cγ, Cα, Cε or Cδ, wherein each of said at least one constant heavy gene when expressed does not express a functional CH1 domain. In further embodiments, VHH single heavy chain locus comprises a camelid VHH, at least one D exon of human origin and at least one J exon and constant region of human origin. It noted that claim 41 is amended to recite a method for the production of a single chain antibody in a mouse comprising expressing a heterologous VHH heavy chain in that mouse specifically in B cells in response to antigen challenge.

The claims are broadly directed to produce single chain antibody in any non human mammal by expressing a chimeric loci comprising VHHat least one Cμ constant heavy chain gene and at least one of Cγ, Cα, Cε, or Cδ constant heavy chain gene which when expressed does not express functional CH1 domains. The breadth of the chimeric VHH loci comprises at least one naturally occurring VHH exon of any known or yet to be discovered specie that do not express functional CH1 or CH4 domains. Thus, breadth of instant claims embraces rearrangement of different naturally occurring VHH coding sequence capable of producing

single heavy chain antibody of different isotype by class switching. Additionally, instant methods read on producing single heavy chain antibody in different non human mammal, wherein post translation machinery that glycosylate the immunoglobulin is know to be specie specific. Further claims as amended read on the a method of providing single chain antibody in any transgenic nonhuman mammal that expresses the VHH locus comprising a regulatory sequence providing expression specifically in B cell by using any method known in prior art. The disclosure provided by the applicant, in view of prior art, must encompass a wide area of knowledge to a reasonably comprehensive extent. In other word each of these, aspect must be shown to a reasonable extent so that one of the ordinary skills in the art would be able to practice the invention without any undue burden being on such Artisan.

The specification contemplates using vector such as YAC or BAC that is suitable of inserting large amounts of nucleic acid, sufficient to encode an entire immunoglobulin heavy chain locus (page 15, lines 14-23). The specification asserts loci and vectors may be introduced into an animal to produce a transgenic animal. It is noted that method of inserting the loci into the genome of a recipient animal will be achieved by microinjection or by introducing DNA into embryonic stem cells (ES) cells which can be inserted into a host embryo to derive transgenic mice (page 27, lines 21-31 bridging to pp 28 see entire section). The specification further contemplates nuclear transfer technique for introducing DNA into any cell. The nuclei of these cells are used to replace the nucleus of a fertilized egg which may be of any species to give rise to transgenic animals (see page 28, lines 3-6). It is noted that specification defines a VHH single chain antibody that does not possess a functional CH1 domain nor a functional CH4 domain (see page 8, lines 22, page 9, line 6-7). The specification also contemplate that the single heavy chain antibody loci have one or more genes which do not express functional CH1 or CH4 domains that may occur by mutation, deletion substituted or other treatment of the CH1 and CH4 exons of the constant heavy region gene (see page 11, lines 27-30).

Applicant's example provides only a schematic of the construct and various techniques without disclosing any specifics of the construct (see page 24) that would have resulted in production of single heavy chain antibody in amount sufficient to be isolate from any non human mammal. It is noted that neither prior art nor instant specification teach any specific VHH loci

that is integrated into the genome of any nonhuman mammal resulting in any production of any functional antibody.

The state of the art at the time of filing of this application recognizes that only IgG2 and IgG3 single heavy chain antibodies are produced in camelids (see Hamers-Casterman et al, Nature, 363:446-448, 1993, art of record). The art teaches that camelid γ2 and γ3 chain are considerably shorter than normal mammalian γ or camel γ1 chains which was found to be absence of CH1 domain (see figure 3 and page 448, col., 1 para. 2). The state of art at the time of filing of this application was generally silent with respect to development of B-cells expressing VHH camelid antibodies (De Genst et al, Dev Comp Immunol. 2006; 30(1-2): 187-98, art of record). These observations suggest that at the time of filing of this application, prior art was generally silent with respect to generating single heavy chain antibody in a non human mammal.

Prior to instant invention it was generally known that humanized antibodies undergo lymphoid-specific gene rearrangement and only a small proportion of mouse B cells expressed the human immunoglobulin chains. The miniloci contributed poorly to serum immunoglobulin which is attributed to competition between the transgenic and endogenous immunoglobulin loci (See Wagner et al abstract Nucleic Acids Res. 1994; 22(8):1389-93 and reference therein). Furthermore, art also teaches low level of B cell expressing immunoglobulin chain is attributed to competition between the introduced minolocus and the endogenous immunoglobulin mouse locus (see Wagner page 1389, col. 2, para. 2). The post filing art Janessens et al (Proc. National Academy of Science, 2006, 15130-15130, art of record) teach using two hybrid chimeric loci in a μMT background mouse that do not produce surface IgM and have a block in B cell development at the pre-B cell stage (see material and method). In the instant case, claims embrace producing single chain heavy antibody by immunizing a nonhuman mammal expressing VHH loci. Given that art teaches that low level of B cell expressing immunoglobulin chain is due to competition between the transgenic and endogenous immunoglobulin loci, instant specification do not enables the breadth of the claims encompassed by a hybrid loci comprising VHH coding sequence of any specie expressed in plurality of different nonhuman mammal. Although, claims as presented do not require isolation of an antibody, the method as claimed

would produce antibody that would compete with endogenous immunoglobulin of the mouse. It is emphasized that competition with the endogenous locus is usually eliminated using Ig knockout strains, in which transgene expression is usually improved. Additionally, art also recognized that poor transloci expression levels are also result of the failure of different sequences to work efficiently in the mouse background. In the instant case, the expression of chimeric loci comprising VHH coding sequence derived form different specie of mammal to produce single heavy chain antibody in response to antigen challenge in plurality of different non human mammal is unpredictable in view of teaching of Wagner et al. One of skilled in art would have to produce different nonhuman mammal to determine the competition between the introduced VHH locus and the endogenous immunoglobulin of the wild type host species without reasonable expectation of success to make use of the invention.

The claims are directed to producing single heavy chain antibody in any transgenic nonhuman mammal. The specification asserts loci and vectors may be introduced into an animal to produce a transgenic animal. It is noted that specification teaches that the method of inserting the loci into the genome of a recipient animal will be achieved by microinjection or by introducing DNA into embryonic stem cells (ES) cells which can be inserted into a host embryo to derive transgenic mice (page 27, lines 21-31 bridging to pp 28 see entire section). However, as stated in previous office action, the state of the art is such that ES cell technology is generally limited to the mouse system and that only putative ES cells exist for other species (Moreadith et al., J. Mol. Med., 1997 p214, abstract, Hochepied et al (Stem Cells, 2004, 22, 441-447; abstract, both art of record). Keefer (Animal Reproduction Science 82-83: 5-12, 2004) recognizes the inefficiency of pronuclear microinjection transgenic techniques and the unpredictability of transgene expression when applied to generating transgenic cows, goats and sheep, for example (see page 6, para. 1, line 1 to page 7, line 4 and also see Houdebine 2000, transgenic Res. 9, 305-320, both art of record). Murray states, "the observation that the oMT 1 a-oGH transgene that is regulated in mice is uncontrollable in both sheep and pigs suggests that transgene constructs still need to be tested in the species of interest." (Murray et al, Theriogenology 51:149-159; pg. 150, para. 4). Additionally, Lillico et al (Drug Discovery Today, 2006, 10(3), 191-196, report " the glycosylation of IgGs in different species highlights the species-specific variation in the sialylation of *N*- and *O*-linked glycans, a major mode of posttranslational glycosylation. IgGs

from some mammals, including cows, sheep and goats, comprise oligosaccharides with *N*-glycolylneuraminic acid (NGNA), whereas other species, including the rabbit (see page 193, col. 2, para. 2). This is further supported by Raju et al (Glycobiology, 2000, 10, 477-486, art of record) who report that the glycosylation of IgG is species specific and reveals a necessity for appropriate cell selection to express rIgGs. Raju et al show that IgG of goat, cow and sheep have different glycosylation pattern that influences their biological function. It is generally known in the art that the activation of effector mechanisms is dependent on structural characteristics of the antibody molecule that result from posttranslational modifications, in particular, glycosylation. Jefferis R (Biotechnol. Prog., 2005, 21, 11-16) while reviewing the state of glyocylation of recombinant antibody states "presence of core oligosaccharide is essential for the expression of IgG-Fc effector functions, and the addition of outer arm sugar residues has a variable influence of the efficacy of specific functions" (see page 15, col. 1, last para.). Jefferis discloses that CHO cell transfected with GNTIII enzyme produced an antibody bearing bisecting GlcNAc residue that exhibited higher activity (page 14, col. 1, lines 1-4) suggesting that glycosylation pattern directly effect antibody function. Wright et al (Trends in Biotechnology, 1997, 26-32) also teach altering amino acid residues by site-directed mutagenesis show mutation of a contact residue for a core N-acetylglucosamine, but not for galactose, resulted in a loss of recognition of the Fc region by Fc receptors (see page 31, col .1, para. 2). These observations clearly show that glycosylation pattern of antibody could directly effect the antibody function. In the instant case, specification is silent with respect to generating single chain heavy antibody in any specie of nonhuman mammal. The guidance provided in post filing art teaches producing a functional single heavy chain antibody in response to an antigen challenge of a transgenic mouse expressing llama VHH loci. In view of foregoing, it is apparent that immunoglobulin produced in the mammal is glycosylated in species specific manner. There is no evidence on record that Vhh heavy chain loci integrated into the genome of any nonhuman mammal would produce an antibody that is functional. Furthermore, Janessens et al show that the chimeric HCAb loci are subject to allelic exclusion, wherein several copies of the transgenic locus can be rearranged and expressed successfully on the same allele in the same cell of the disclosed transgenic mouse. However, it is generally known that evidence of allelic exclusion mostly exit in mouse and human. There is no evidence that same could be extrapolated to any other nonhuman mammal.

In view of foregoing, it is apparent that an artisan would have to perform undue experimentation to test different VHH loci comprising naturally occurring VHH coding sequence form other specie in plurality of different nonhuman mammal. The specification fails to correlate specific elements of the naturally occurring VHH loci comprising VHH coding sequence of any other specie that may result in formation of single heavy chain antibody in response to challenge to antigen. The specification does not provide sufficient guidance to overcome the unpredictability for of specie specific glycosylation of immunoglobulin and allelic exclusion in practicing the claimed method in any nonhuman mammal. Thus, skilled artisan would have to empirically test the method by making VHH loci with plurality of different elements and then test each one of them that reliably express in a non human mammal to generate and antibody in response to an antigen and then characterize any resulting single heavy chain antibody that may be complexed with animals' endogenous antibody to make use of any such antibody without reasonable expectation of success as supported by the observations in the art record.

In the instant case, the specific elements contemplated by the specification and enabled by post filing art of Janessens et al (Proc. National Academy of Science, 2006, 15130-15130, art o record) in the construction of vector comprising llama VHH coding sequence for generating single heavy chain antibody in the transgenic mouse were derived from the prior art based on reports of their function in mice. Absent of evidence to the contrary, it is not clear that any other naturally occurring VHH coding sequence of any other specie would be functional in other animal species in the same manner as they have been demonstrated in the transgenic mouse. Thus, the art of record at the time of the invention does not provide enabling support for the claimed invention of a method of using nonhuman mammal to generate single heavy chain antibody commensurate with full scope of the claims. An artisan would have to perform undue experimentation to express the VHH exon of other specie in B cells of different species as embraced by the breadth of instant claims to make and use the invention. In conclusion, in view of breadth of the claims and absence of a strong showing by Applicant, in the way of specific guidance and direction, and/or working examples demonstrating the same, such invention as claimed by Applicant is not enabled commensurate with the scope of the claimed inventions.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim34-36 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 34-36 are vague and indefinite to the extent they depend on the canceled claim 3. It is noted that claim 34-36 recites the limitation "wherein the camelised VH single heavy chain" that is not required by any of the pending claims. Appropriate action is required.

New-Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 7, 10-11, 39, 41, 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Surani et al (US Patent no 5545807, dated 8/13/1996), Lonberg et al (US Patent no 5625126, dated 4/29/1997, art of record), Nguyen et al (Molecular Immunology, 1999, 515-524) and NCBI accession no. (AF305944, dated 3/15/2001).

With respect to claim 1, 7, 10-11, 39, 41, 43, Surani et al teach a method to produce an antibody in a transgenic mouse comprising a heterologous chimeric construct that generate antibody specifically in B cell of said mouse in response to antigen challenge, wherein the hybrid unrearranged immunoglobulin heavy chain gene comprising a mouse VH gene segment, a human VH gene segment, human and mouse D gene segments, human J gene segments, and a gene segment encoding a human mu constant effector region (see col. 3, lime 7-16 and col. 4, line 9-47). It is noted that Surani et al also teach that the vector also contains the necessary IgH enhancer required for B-cell specific expression (see col. 14, line 37). Surani et al teach immunizing said mouse to establish hybridoma which produced antibodies comprising human heavy chain and mouse light chain (example 3 and claim 5 of '807). While, Bruggemann and

teach that hybrid loci comprising different element from different species are functional, but differ from claimed invention by not explicitly teaching construct generating antibody of different isotype by class switching.

However, such was known in prior art. For instance, Lonberg et al ('126) teach a method to induce heterologous antibody production of various isotypes, including: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgA, IgD, of IgE (col. 4, line 9-10) in the transgenic mouse (col. 2, lines 59-60). It is noted that the method involves undergoing isotype switching that occurs by recombination events which involve at least one switch sequence region in the transgene (col. 4, lines 34-40). Furthermore, the recombination of variable region gene segments to form functional heavy and light chain variable regions is mediated by recombination signal sequences (RSS's) that flank recombinationally competent V, D and J segments. Lonberg disclose that these sequences are found on the J, or downstream side, of each V and D gene segment (col. 22, lines 22-45). Lonberg also disclose mice that produce B cells is also capable of alternatively expressing antibodies comprising fully human heavy chains and antibodies comprising chimeric (human variable/mouse constant) heavy chains, by trans-switching (col. 43, lines 3-45). Lonberg discloses that the immunoglobulin heavy chain transgene could comprise one or more of each of the VH, D, and JH gene segments and two or more of the CH genes. It is noted that with regard to the CH segments for the heavy chain transgene, it is preferred that the transgene contain at least one μgene segment and at least one other constant region gene segment, more preferably a γ gene segment. Lonberg discloses that switch regions can be linked upstream heavy chain C gene that do not naturally occurs next to a particular switch region (col. 33, lines 1-6). It is also noted that Lonberg contemplate a C2 segments that is preferably a human γ1 or γ 3 genes. Lonberg also discloses that murine γ2a and γ2b can also be used, as may downstream (i.e., switched) isotype genes form various other species (col. 34, lines 19-24). Lonberg teaches that the switch regions used in the transgene are preferably murine or human (col. 33, lines 62-67). In addition, Lonberg also discloses a vector pGP1h that includes the promoter leader sequence exon (example 18). However, Lonberg et al (126') do not disclose a transgene wherein VH region comprises a naturally occurring VHH region that produces a single chain heavy antibody devoid of light chain.

The deficiency of Surani et al and Loneberg is cured by Nguyen who teaches that heavy-chain antibodies (HCAbs) lack the segments first domain of the constant region (CH1), which is present in the genome but is spliced. It is disclosed that loss of the splice consensus signal is responsible for the removal of the entire CH1 domain in camel g2a heavy-chain Immunoglobulins (see abstract). Nguyen et al also teach complete nucleotide (nt.) sequence of the camel g2a heavy-chain constant gene obtained from a liver genomic library (see, page 516, col. 2, para. 2). NCBI accession no provided the germ-line VHH coding sequence.

Thus, based upon the need to enlarge the primary Ag-binding repertoire of the HCAb using a VHH coding sequence taught by NCBI accession no and in view of detailed guidance provided in the Loneberg and Surani et al specification for switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a mouse for generating heterologous antibody in a mouse, it would have been *prima facie* obvious to the skilled artisan to modify the method of Surani et al by substituting the VH gene segment with a VHH exon comprising naturally occurring VHH coding sequence disclosed by Nguyen et al/NCBI accession number with reasonable expectation of success. A person of skill in the art would have been motivated to substitute human or mouse VH gene segment disclosed by Surani et al with VHH coding sequence disclosed by NCBI accession, as a matter of design choice, said design choice amounting to combining prior art elements according to known methods to yield predictable results. One of ordinary skill in the art would have been motivated to produce heterologous antibody in a mouse by further optimizing and changing constant heavy chain gene from other species such as mouse and rabbit. Lonberg had already disclosed that switch region from different isotype can be operatively linked to a particular constant region, the skilled artisan would have been motivated to use either a switch region of same isotype or a switch region of different isotype to promote class switch to any particular constant region gene. Thus in view of detailed teaching of Lonberg as discusses above, it would have been *prima facie* obvious to the skilled artisan at the time of filing to modify the switch region of different species as taught by Lonberg. Furthermore, Nguyen et al had already described that heavy-chain antibodies (HCAbs) lack the segments of first domain of the constant region (CH1), thus one of ordinary skill would be motivated to splice CH1 domain such that constant heavy chain gene when expressed does not express a functional CH1 domain to produce only single heavy chain antibody devoid of

light chains. One of ordinary skill in the art would be motivated to do so as it was known in the art that single chain heavy antibody recognizes broad range of epitope some of which differ form the conventional antibodies. Other limitations of wherein constant heavy chain are from different specie of mammal was routine optimization and was well known in the art and therefore obvious variables when optimizing to use either a constant region of same specie or a constant region of different species in view of Green. Further in view of the high level of skill in molecular biology techniques at the time of filing, one of the ordinary skills in the art would expect a reasonable expectation of success in modifying the transgene by substituting VHH coding sequence in the method disclosed by Surani et al for producing heterologous antibody of different isotype using the method disclosed by Loneberg in mouse such that functional CH1 domain in the constant heavy chain gene is not expressed. One of ordinary skill in art would have been motivated to combine the teaching of Surani et al, Loneberg, Nguyen et al and NCBI accession number because the method would have yielded a single heavy chain VHH antibody for therapeutic use.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Should the claims be amended to state wherein CH1 domain is deleted from each of said at least one constant heavy chain region, and isolating single heavy chain antibody of plurality of different classes against said antigen from said mouse, the above obviousness rejection may be overcome pending further consideration.

Claim 33 is rejected under 35 U.S.C. 103(a) as being unpatentable over Surani et al (US Patent no 5545807, dated 8/13/1996), Lonberg et al (US Patent no 5625126, dated 4/29/1997), Nguyen et al (Molecular Immunology, 1999, 515-524, IDS) and NCBI accession no. (AF305944, dated 3/15/2001) as applied to claims 2, 7, 10-11, 39, 41 and 43 above, and further in view of O'Keefe et al (US 20020147312, dated 10/10/2002, effective filing date 2/2/2001).

The reference of Surani et al, Lonberg et al (US Patent no 5625126, dated 4/29/1997), Nguyen et al and NCBI accession no have been described before and relied in same mummer here. While combination of references teach a method for producing a single heavy chain

antibody in a transgenic mouse comprising expressing a heterologous VHH heavy chain locus, but differ from claimed invention by not disclosing entire VHH coding single chain locus is of camelid origin.

However, such was known in prior art. For instance, O'Keefe et al teaches constant region of antibody can be replaced with another constant region using techniques known in art. Specifically, O'Keefe et al disclose that constant region of other species including rodent, camel and rabbit are known in art and routinely used to produce antibody (see page 11, para. 18 and reference cited therein).

It would have been obvious for one of ordinary skill in the art at the time of invention to combine the respective teachings to modify the method by using substituting constant chain regions from one species disclosed by Surani et al/ Lonberg et al with another from camel using r known method to produce single heavy chain antibody. A person of skill in the art would have been motivated to substitute constant chain of one specie with another such as camel, as a matter of design choice, said design choice amounting to combining prior art elements according to known methods to yield predictable results. One who would have practiced the invention would have had reasonable expectation of success since O'Keefe et al had already disclosed that constant region of different species including rodent, camel and rabbit were routinely used to produce antibody, while Surani et al, Loneberg and Nguyen taught method of generating single heavy chain antibody. Thus, it would have only required routine experimentation for one of ordinary skill in the art to isolate antibody in the method disclosed by Surani et al, Loneberg and Nguyen using the constant region from camel origin as required by the instant claims.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claim 44 is rejected under 35 U.S.C. 103(a) as being unpatentable over Surani et al (US Patent no 5545807, dated 8/13/1996), Lonberg et al (US Patent no 5625126, dated 4/29/1997, art of record), Nguyen et al (Molecular Immunology, 1999, 515-524, IDS) and NCBI accession no. (AF305944, dated 3/15/2001).as applied to claims 1, 7, 10-11, 39, 41, 43 above, and further in view of Davies et al (Protein Eng. 1996, 531-537, IDS).

The reference of Surani et al, Loneberg, Nguyen et al and NCBI accession no have been described before and relied in same mummer here. While combination of references teach a method for producing a single heavy chain antibody in a transgenic mouse comprising expressing a heterologous VHH heavy chain locus, but differ from claimed invention by not disclosing isolating various variable fragments using phage display.

However, such was known in prior art. For instance, Davies teaches a method to isolate antigen specific VH domain using phage display (see page 532, col. 1, para. 2).

It would have been obvious for one of ordinary skill in the art at the time of invention to combine the respective teachings to modify the method of Surani et al. Loneberg and Nguyen et al with another known method to isolate antigen specific VH domain using phage display as disclosed by Davies et al. A person of skill in the art would have been motivated to isolate variable region fragment using phage display disclosed by Davies, as a matter of design choice, said design choice amounting to combining prior art elements according to known methods to yield predictable results. One who would have practiced the invention would have had reasonable expectation of success since Davies et al had already disclosed that phage display could be used to isolate variable region fragment, while Surani et al and Nguyen taught method of generating single heavy chain antibody. Thus, it would have only required routine experimentation for one of ordinary skill in the art to isolate antibody in the method disclosed by Surani et al and Nguyen using the known phage display methods such as required by the instant claims.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Conclusion

No claims allowed

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

Xu et al (Immunity 2000 13, 37-45) show that the presence of one single V gene is sufficient to obtain function antibody.

Green et al (20030093820, dated 11/30/2001, art of record)

Riechmann et al (J Immunol Methods. 1999; 231(1-2): 25-38, art of record).

Imam et al (2000) *Nucleic Acids Res* 15, E65.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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